The Role of the *MATE45* Transporter in the Abiotic Stress Response of *Arabidopsis thaliana*

Undergraduate Research Thesis

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ABSTRACT

A plant's healthy growth and development is dependent on the plant's ability to transport its metabolites to the location where they carry out their function. These metabolites include hormones, which have a large role in the growth and development of the plant in normal conditions and also in conditions of abiotic stress. For example, abscisic acid (ABA) is a hormone that has a role in drought and salt stress, by inducing the genes that encode the dehydration tolerance proteins and ion channel proteins that are involved in changing the guard cell shape that leads to stomata closure. This thesis focuses on the role of a specific Multidrug and Toxic Compound Extrusion (MATE) transporter, MATE45, which we found has a role in the ABA pathway in *Arabidopsis thaliana*. *mate45-1* is a mutant of *MATE45* with a T-DNA insertion, which lead to an overexpressed transcript of the gene, but created a truncated gene product. Seedlings of *mate45-1* grown in anthocyanin induction condition (AIC; 3% sucrose in water) stress showed a failure to arrest growth phenotype that was recessive. The WT phenotype was rescued in *mate45-1* when a functional copy of *MATE45* was inserted. This allowed us to confirm that the *mate45-1* phenotype we observed was indeed due to a mutation in the *MATE45* gene. The *mate45-1* phenotype was similar to that of a plant in which the *MATE45* gene had been silenced. This indicated that the truncation of the *mate45-1* mutant gene made a product that had reduced function, similar to the scenario where the transporter was being silenced. The *mate45-1* phenotype was not dependent on the presence or absence of anthocyanins in the plant, but was dependent on ABA. Overall, this showed that *MATE45* did indeed have a role in the plant's response to abiotic stress, which affected the ABA pathway.

INTRODUCTION

Plants must be able to respond to their ever-changing environments in order to survive exposure to abiotic stresses. With the effects of global climate change, plants that are not able to rapidly respond to environmental changes may suffer reduced yields, which can negatively affect food production worldwide (Rosenweig and Parry, 1994). When subjected to cold, salt, sugar, or acidic stress, *Arabidopsis thaliana* has been shown to have a different composition of anthocyanins, which vary depending on the stress condition (Kovinich et al., 2014). Anthocyanins are secondary plant metabolites that give plants much of their red or purple color. There is a large diversity of anthocyanins, as they are readily modified by glycosylation, methylation, and acylation enzymes (Holton and Cornish, 1995). The protective functions of anthocyanins in plants include reduction of photoinhibition during conditions of high light (Field et al., 2001), prevention of DNA damage from ultraviolet radiation (Stapleton and Walbot, 1994), and elimination of reactive oxygen species (Gould et al., 2002).

Just like with anthocyanins, hormones are present in typical growth conditions, but the composition varies depending on the growth stage and environmental conditions. Abscisic acid (ABA) is a hormone that is both involved in the regulation of many aspects of plant development including seed germination, leaf organogenesis, and cell expansion (Barrero et al., 2005), and is also involved in the regulation of stress response under drought and salt conditions (Zhu, 2002). Additionally, ABA has been found to promote anthocyanin accumulation in grapevine (*Vitis vinifera*) cell cultures (Gagne, 2011), and *Arabidopsis* seedlings (Loreti et al., 2008). ABA-deficient mutants of *Arabidopsis* are smaller than WT plants under 'non-stressful' growth conditions. However, under drought and salt stresses, they perform

poorly compared to WT because they fail to induce genes that encode dehydration tolerance proteins and ion channel proteins that are involved in changing the guard cell shape that leads to stomata closure (Zhu, 2002). ABA is transported across the plasma membrane of vascular cells to enable transit to guard cells by the protein ABCG25. If the *ABCG25* gene is overexpressed, there is an over-accumulation of ABA, which induces stomatal closure, leads to slower water loss during dehydration, and therefore increases protection against drought (Kuromori et al., 2010).

Recent studies have shown that a plant's ability to respond to abiotic stresses is dependent on the proper function of its metabolite transporters. The Multidrug and Toxic Compound Extrusion (MATE) transporters are antiporters driven by either Na^+ or H^+ gradients in order to transport a wide range of substrates (Kuroda and Tsuchiya, 2009). For example, in grapevine, two genes from the MATE-family, *anthoMATE1* (*AM1*) and *AM3*, have a role in the transport of acylated anthocyanins across the tonoplast into the vacuole where they are stored (Gomez et al., 2008). In addition, EDS5 is a MATE transporter in *Arabidopsis*, which transports salicylic acid (SA) out of the chloroplast, the site of SA synthesis, when the plant is exposed to pathogens or UV-C light. If there is a null mutation of this transporter, the plant accumulates very little SA and is hypersusceptible to this kind of damage (Serrano et al., 2013; Nawrath et al., 2002). Furthermore, a MATE protein called AtDTX50 is an ABA efflux transporter also found in *Arabidopsis*. It is mainly expressed in vascular tissues and guard cells, and *dtx50* mutants are more sensitive to ABA in growth inhibition and more tolerant to drought conditions, because ABA is retained in the guard cells (Zhang et al., 2014).

In order to find transporters that are involved in the response to abiotic stress, we performed a screen of MATE transporter mutants grown under anthocyanin induction condition (AIC; 3% sucrose in water). *Arabidopsis* produces a high level of anthocyanins when grown in AIC, with an anthocyanin profile similar to that produced under salt stress (Kovinich et al., 2014). Since the production of anthocyanins is a common response to abiotic stresses, we hypothesized that we could identify mutants of transporter genes that are involved in the response to abiotic stress by visually screening lines for reduced levels of anthocyanins when grown in AIC. The following thesis describes the genetic analysis of one of the genes that we found in our screen, *MATE45*. We genetically characterized *MATE45* and the role that it played in the plant's response to abiotic stress. We accomplished this by genotypically and phenotypically analyzing *mate45* mutants and comparing them to wild type *Arabidopsis*.

MATERIALS AND METHODS

Plant materials

The wild-type seeds of *Arabidopsis thaliana* are ecotype Columbia. The crossed lines (*mate45-1 tt4-11* and *mate45-1 aba2-1*), silenced lines (*siMATE45-30* and *siMATE45-31*), heterozygous line (*mate45*(+/-)), and complemented line (*MATE45 mate45-1*) were provided by Dr. Nik Kovinich.

Anthocyanin induction condition (AIC)

The seeds were sterilized for 5 minutes in 70 % ethanol/0.2 % Triton X, and rinsed 3 times with 95 % ethanol. Approximately 120 seeds were transferred into a 35 mm diameter petri dish using a custom scoop, and allowed to dry. The petri dish was filled with 3.5 mL of sterile water with 3% sucrose, and was placed in 4 °C. After 3 days, the seeds were transferred to light (85-100 μmol m^{-2} s^{-1}) on a rotary shaker at 100 rpm in 22 °C and grown for at least 10 days until growth had arrested. In some experiments, compounds were added to the seedlings as they were growing. Either 3μM 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA), N-1-naphthylphthalamic acid (NPA), 10 μM of fluridone, or equal volume of solvent DMSO was added to the petri dish. These compounds were added three days after germination, and then the seedlings were incubated in AIC until growth had arrested. This was performed by me.

Growth on soil

Plants that were grown on soil were allowed to dry immediately after sterilization and were then planted on half strength Murashige & Skoog medium (Murashige and Skoog, 1962) containing 3 % sucrose (w/v) and 0.5 % agar (w/v). They were placed in 4 °C for three days and then transferred to 22 °C 24h light, until they developed true leaves. Then they were

transplanted onto Sunshine LC1 Professional Growing Mix, where they grew in 22 °C 16 h light/8 h dark. This was performed by me.

Gene expression analysis

The total RNA was isolated using a Sigma Aldrich Spectrum Plant Total RNA Kit. At least three biological replicates were taken from each line. The seedlings were grown in AIC and harvested 4 days after germination, before the development of any primordia. One microgram of RNA was treated with DNAse I (Amplification Grade, Life Technologies) according to the instructions. The RNA was then reverse transcribed using SuperScript II to make cDNA, and RT-PCR or qRT-PCR was performed using primers in Table 1. PCR amplicons were isolated from an agarose gel and cloned into vector pDONR221 using primers 221AT1a/221AT1r and BP clonase. The clones were sequenced using primers shown in Table 1 and Figure 1. The RNA extraction, DNAse treatment, and cDNA synthesis were performed by me. The qRT-PCR, cloning, and data analysis were performed by Dr. Kovinich.

Target	**Primer**	**Sequence (5' to 3')**
ACTIN2	qACTf	ACCAGCTCTTCCATCGAGAA
ACTIN2	qACTr	GGGCATCTGAATCTCTCAGC
MATE45 and mate45-1	1 (qAT1f)	CTGCCACTGGGATTTTGATT
MATE45 and mate45-1	2 (qAT1r)	CGGCTAGTCTGGCCTTGTAA
mate45-1	3 (q865f)	AGCCTCCTTTGGATCAACCT
mate45-1	4 (L4n)	GTAGATTTCCCGGACATGAAG
MATE45	6 (AT1x2r)	TCAGTCTCATTGCCTTCACG
MATE45	7 (qAT1nf)	TGGGTTTAGTGGGTTGTGGT
MATE45	8 (qAT1nr)	CGTCAGTCTCATTGCCTTCA

Table 1. The sequence of the primers used to measure the expression of *MATE45* and *mate45-1*. The target describes what specific transcript or mutant of the transcript was amplified. The number of the primer corresponds to the numbers shown in Figure 1.

Primordia counting

The true leaf primordia were counted under a Nikon SMZ1500 microscope with 10x eyepiece magnification and 0.75x objective magnification (7.5x total magnification). Each seedling was analyzed for either the presence or the absence of visible true leaf primordia after more than 10 days growth in AIC to ensure growth had arrested. This was performed by me.

Dehydration

The plants were grown on soil as described above, and then the rosette was cut at its base and placed on the bench top to dry. The mass of each rosette was measured immediately and every subsequent hour for the next 5 hours. The percentage of water loss was calculated by the following formula: $\text{water loss} = \left(\frac{\text{mass at 0h} - \text{mass at 5h}}{\text{mass at 0h}}\right) \times 100$. The measurements were taken by me, while the calculations were performed by Dr. Kovinich.

RESULTS

Analysis of *mate45-1* gene expression levels

The first question we sought to answer was how the *mate45-1* mutation affected the *MATE45* gene. We compared the expression levels of various segments of the gene between WT and mate45-1. Figure 1 shows the location of the primers on *MATE45*.

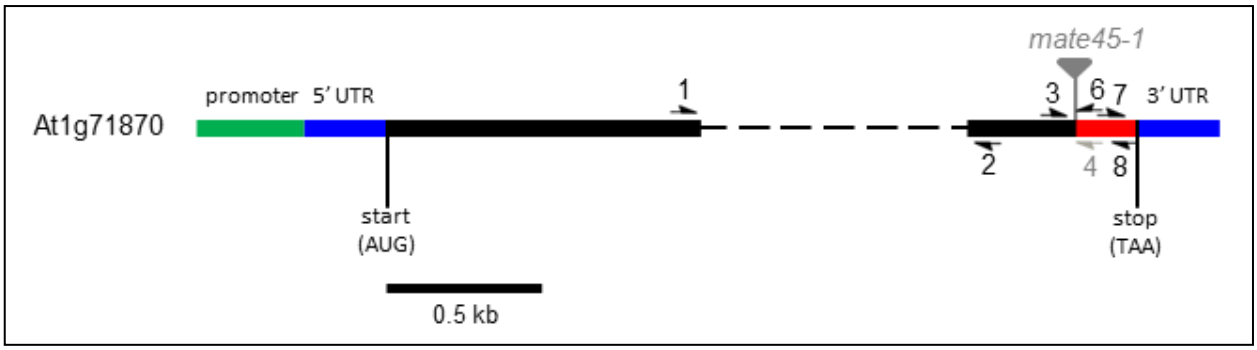

Figure 1. At1g71870 shows the *MATE45* gene with the intron (dashed line) and the exons (solid black lines). The size and location of the intron is drawn to scale; however, the sizes of the promoter and the untranslated regions are unknown and are therefore not drawn to scale. The red bar shows the portion that is truncated in *mate45-1*. The numbered half arrows show the position of the primers that were used (Table 1). The black primers bind to the gene, while the gray primer binds to the T-DNA insertion. The location of the T-DNA insertion is labeled *mate45-1*.

In Figure 2, *ACTIN2* was used as a control, because it is expressed regardless of the *mate45-1* mutation. The presence of a band with primers 1-2 for both *mate45-1* and WT shows that the region upstream of the T-DNA insertion is unaffected in *mate45-1*. Primers 3-4, which bind the *MATE45* transcript and T-DNA sequence, respectively, amplify *mate45-1* cDNA but not the WT template. This showed that *mate45-1* transcripts coded T-DNA sequence. In contrast, there was not a band for *mate45-1* with the primers 7-8 that bind *MATE45* downstream of the *mate45-1* T-DNA insertion site, but there is a band for WT. This showed that the T-DNA insertion in *mate45-1* is transcribed and that T-DNA sequence replaces a segment of *MATE45* transcript sequence in *mate45-1*.

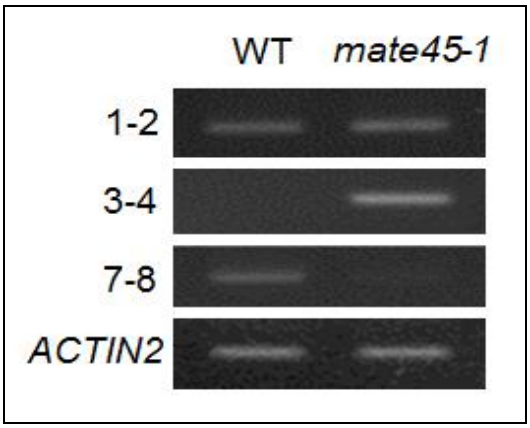

Figure 2. qRT-PCR of *MATE45* cDNA for WT and *mate45-1*. The positions of the primers are shown in Figure 1. The *ACTIN2* primers were used as a control.

The T-DNA insertion of *mate45-1* coded a stop codon that caused a truncation of the MATE45 protein. Sequencing the cDNA of *MATE45* showed this. Figure 3 shows an amino acid sequence alignment for WT and *mate45-1*, in which *mate45-1* is missing the final 61 amino acids. Figure 4 shows a predicted transmembrane protein model of WT and *mate45-1*. Based on this model, much of the predicted protein topology of *mate45-1* was altered because of the 61 amino acid deletion. This suggests that the function of the protein may have been altered, as a result.

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CLUSTAL O(1.2.1) multiple sequence alignment


WT            MEDKIQSDDFTSHKNPTLPQVIEELKELWAMVLPITAMNCLVYVRAVVSVLFLGRLGSLE      60
mate45-1      MEDKIQSDDFTSHKNPTLPQVIEELKELWAMVLPITAMNCLVYVRAVVSVLFLGRLGSLE      60
              ************************************************************

WT            LAGGALSIGFTNITGYSVMVGLASGLEPVCSQAYGSKNWDLLTLSLHRMVVILLMASLPI      120
mate45-1      LAGGALSIGFTNITGYSVMVGLASGLEPVCSQAYGSKNWDLLTLSLHRMVVILLMASLPI      120
              ************************************************************

WT            SLLWINLGPIMLFMGQNPEITATAAEYCLYALPDLLTNTLLQPLRVYLRSQRATKPMMWC      180
mate45-1      SLLWINLGPIMLFMGQNPEITATAAEYCLYALPDLLTNTLLQPLRVYLRSQRATKPMMWC      180
              ************************************************************

WT            TLAAVAFHVPLNYWLVMVKHWGVPGVAIASVVTNLIMVVLLVGYVWVSGMLQKRVSGDGD      240
mate45-1      TLAAVAFHVPLNYWLVMVKHWGVPGVAIASVVTNLIMVVLLVGYVWVSGMLQKRVSGDGD      240
              ************************************************************

WT            GGSTTMVAVVAQSSSVMELVGGLGPLMRVAVPSCLGICLEWWWYEIVIVMGGYLENPKLA      300
mate45-1      GGSTTMVAVVAQSSSVMELVGGLGPLMRVAVPSCLGICLEWWWYEIVIVMGGYLENPKLA      300
              ************************************************************

WT            VAATGILIQTTSLMYTVPMALAGCVSARVGNELGAGRPYKARLAANVALACAFVVGALNV      360
mate45-1      VAATGILIQTTSLMYTVPMALAGCVSARVGNELGAGRPYKARLAANVALACAFVVGALNV      360
              ************************************************************

WT            AWTVILKERWAGLFTGYEPLKVLVASVMPIVGLCELGNCPQTTGCGILRGTGRPAVGAHV      420
mate45-1      AWTVILKERWAGLFTGYEPLKVLVASVMPIVGLCELGNCPQTTGCGILRGTGRPAVGAHV      420
              ************************************************************

WT            NLGSFYFVGTPVAVGLAFWLKIGFSGLWFGLLSAQAACVVSILYAVLARTDWEGEAVKAM      480
mate45-1      NLGSFYFVGTPVAVGLAFWLKIGFSGLWF------------------------------      449
              ****************************

WT            RLTSLEMRKVGQDEESSLLLLDDEKLGDVL    510
mate45-1      ------------------------------    449
```

Figure 3. An amino acid sequence alignment of WT and *mate45-1* made with Clustal Omega (Sievers et al., 2011; Goujon et al., 2010). The asterisks indicate matching amino acids, and the dashes indicate missing amino acids.

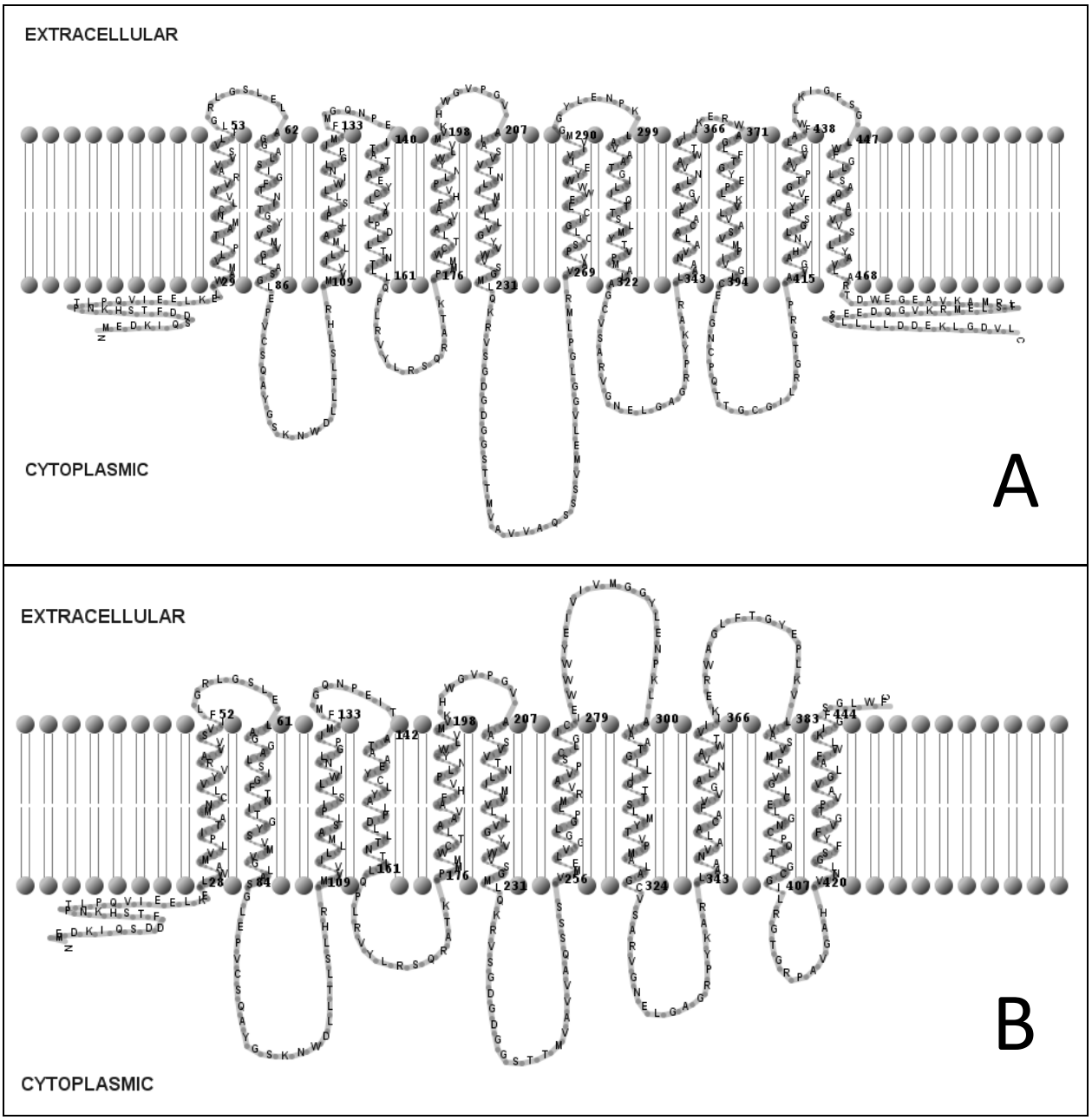

Figure 4. A transmembrane protein model of WT MATE45 (part A) and the truncated *mate45-1* (part B). The images were generated using HMMTOP (Tusnady and Simon, 1998; Tusnady and Simon, 2001) and TMRPres2D (Spyropoulos et al., 2004).

To measure the expression levels of the *MATE45* transcript, we performed quantitative reverse transcriptase PCR (qRT-PCR). The expression levels were measured four days after germination. According to Figure 5, the relative gene expression of *MATE45* relative to *ACTIN2*

is significantly increased in *mate45-1* whenever the primers were placed before the site of the mutation. However, the relative gene expression is about zero when the primers were placed after the site of mutation. This shows that *mate45-1* causes the *MATE45* gene to be overexpressed at this growth stage, but the transcripts are truncated.

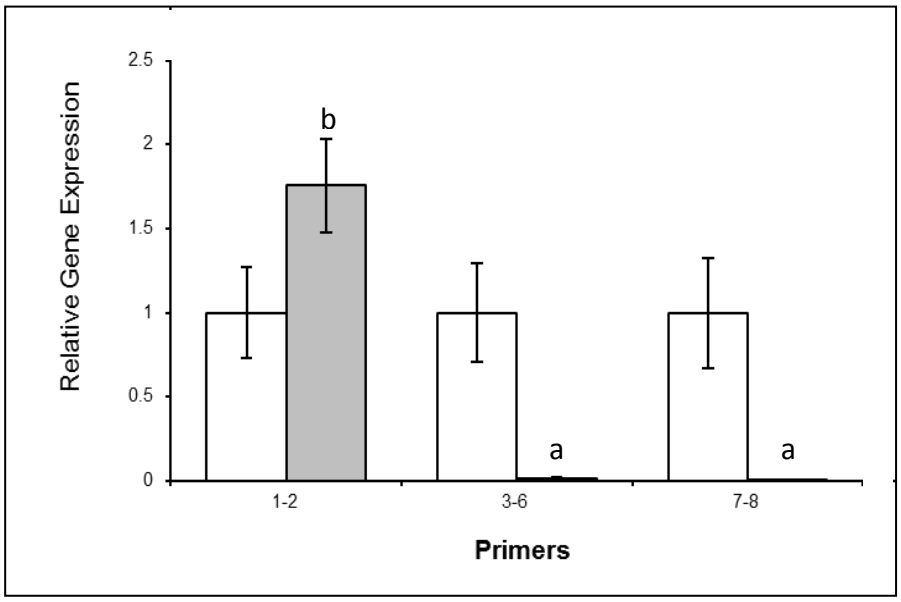

Figure 5. The relative gene expressions of *MATE45* four days after germination, using various primers in WT (white bar) and *mate45-1* (gray bar). The positions of the primers are shown in Figure 1. *MATE45* expression is compared to *ACTIN2*, and then the WT levels are all normalized to 1. The bars are denoted by "a" if significantly lower than WT and "b" if significantly higher than WT, based on a Student's t-test ($p < 0.05$).

Growth response to AIC stress

One of the ways that the mutation affected the phenotype of *mate45-1* was by failing to arrest growth of true leaf primordia under the stress of anthocyanin induction condition (AIC). We chose to analyze this phenotype because it showed that MATE45 had a role in coordinating the seedling's stress response with development. The penetrance of this phenotype could be quantified by counting the number of seedlings that had visible true leaf primordia, compared to the total number of seedlings. Figure 6 shows an example of a seedling with primordia and a seedling without primordia.

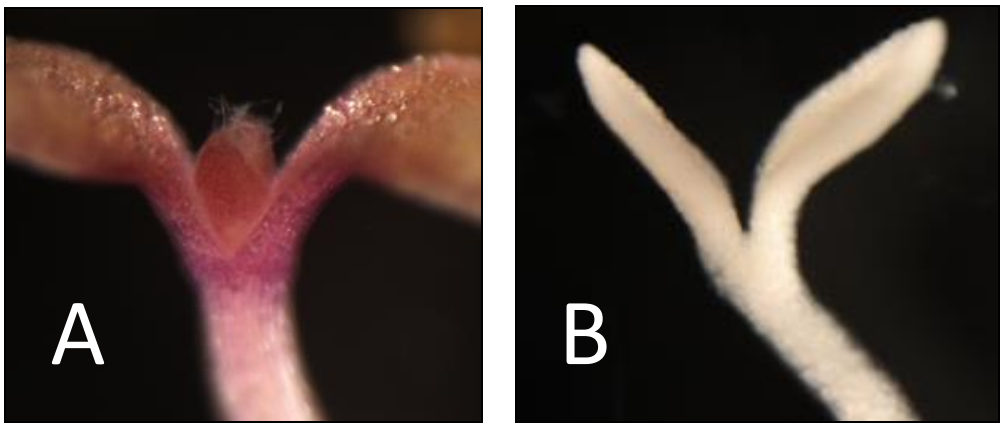

Figure 6. An image of a *mate45-1* seedling with primordia (part A) and a WT seedling without primordia (part B). Both seedlings were grown under AIC stress. The images are at 90x magnification.

We first observed this failure to arrest growth phenotype in the ABA transporter mutant, *abcg25-5*, and also in *mate45-1*. According to Figure 7, both mutants failed to arrest growth of their true leaf primordia at greater frequencies compared to WT. Since *mate45-1* overexpressed truncated transcripts of the *MATE45* gene, we wanted to determine whether *mate45-1* is dominant or recessive. Figure 7 shows that when the *MATE45* gene was heterozygous (*mate45-1* (+/-)), the percentage of seedlings with primordia was similar to WT levels. This suggests that the *mate45-1* mutation is recessive. Next, we wanted to see if we could achieve the *mate45-1* phenotype by silencing the *MATE45* gene in a WT background. Figure 8 shows the reduced expression levels of *MATE45* compared to *ACTIN2* in the silenced lines, *siMATE45-30* and *siMATE45-31*. There was still some expression of *MATE45* for the silenced lines, as compared to *mate45-1*, which had almost no expression. Nevertheless, the silenced lines had a percentage of seedlings with primordia that was similar to *mate45-1*. This suggests that the truncation of *mate45-1* renders the gene ineffective, as if it were silenced. When the *mate45-1* mutant was transformed with a functional copy of the *MATE45* gene (*MATE45 mate45-1*), the percentage of seedlings with primordia was similar to the WT

percentage. The fact that we were able to complement the mutation shows that it was caused by a dysfunctional *MATE45* gene.

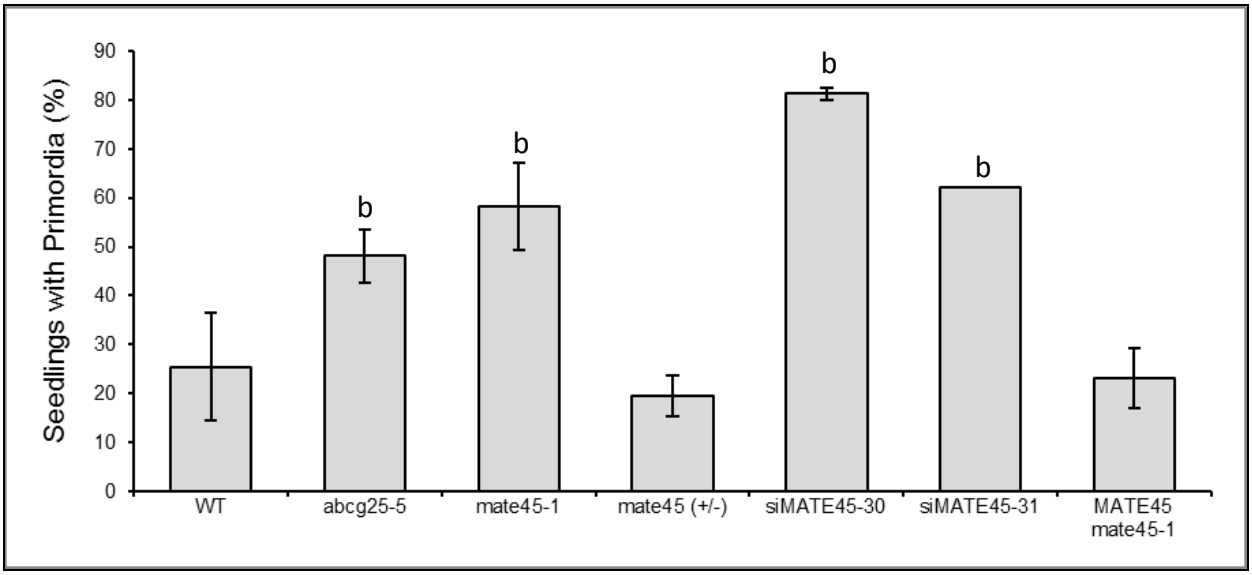

Figure 7. The percentage of seedlings with primordia when grown under AIC stress. WT is the wild type and *mate45-1* is the mutant for the *MATE45* gene. The a*bcg25-5* is an ABA transporter mutant. *mate45* (+/-) is heterozygous for the *mate45-1* mutation. *siMATE45-30* and *siMATE45-31* are lines in which the *MATE45* gene has been silenced in a WT background. *MATE45 mate45-1* is a *mate45-1* mutant in which a functional copy of *MATE45* has been inserted. The bars are denoted by "a" if significantly lower than WT and "b" if significantly higher than WT, based on a Student's t-test ($p < 0.05$).

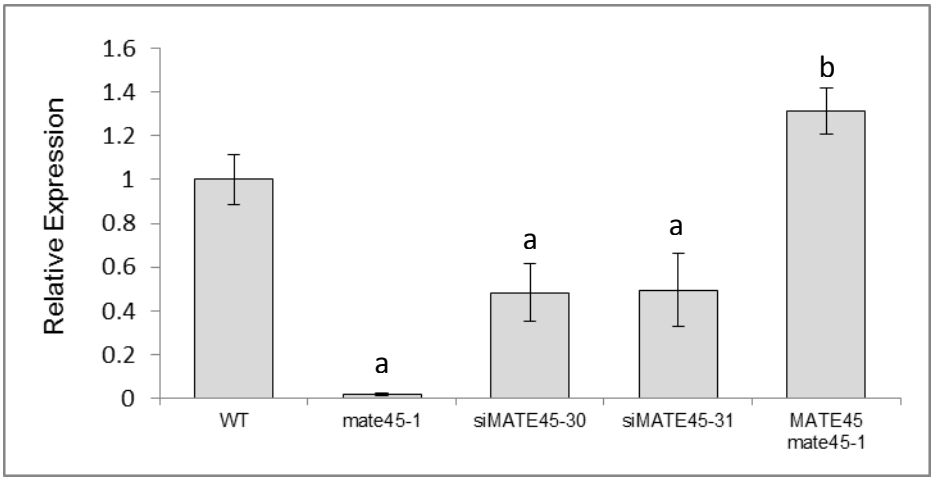

Figure 8. The relative expression of *MATE45* compared to the expression of *ACTIN2*, measured with primers 7-8 (Table 1 and Figure 1). The WT expression level has been normalized to 1. The bars are denoted by "a" if significantly lower than WT and "b" if significantly higher than WT, based on a Student's t-test ($p < 0.05$).

After seeing that *MATE45* had some role in stress response, we wanted to know whether or not the phenotype was affected by anthocyanins. This is because anthocyanins are also thought to have a role in stress response, and so it was possible that the pathways were somehow related. To accomplish this, we crossed *mate45-1* with *tt4-11*, an anthocyanin synthesis mutant. Figure 9 shows that the *tt4-11* line had the same percentage of seedlings with primordia as WT, while the *mate45-1* x *tt4-11* line had the same percentage of seedlings with primordia as *mate45-1*. This demonstrates that *mate45-1* is not affected by the presence or absence of anthocyanins.

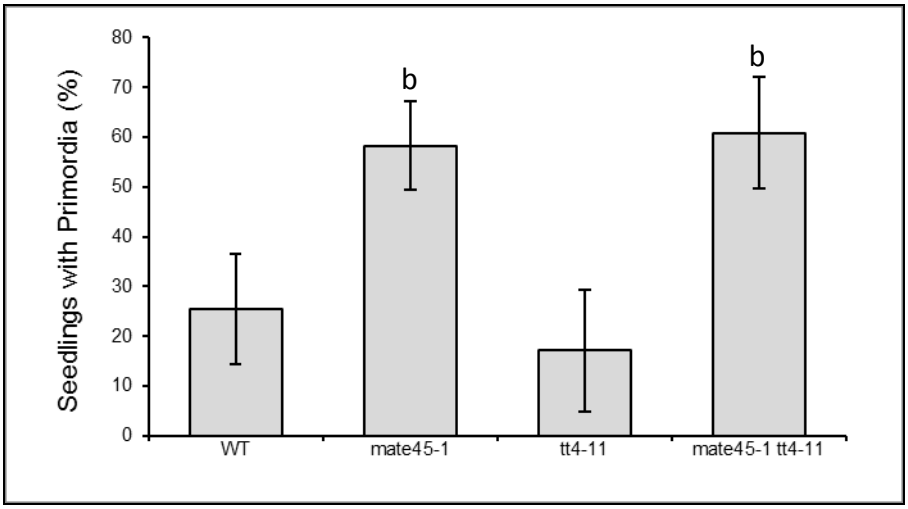

Figure 9. The percentage of seedlings with primordia when grown under AIC stress. *tt4-11* is an anthocyanin synthesis mutant. *mate45-1 tt4-11* is a cross that is homozygous for both the *mate45-1* and *tt4-11* mutations. The bars are denoted by "a" if significantly lower than WT and "b" if significantly higher than WT, based on a Student's t-test ($p < 0.05$).

ABA is known to be required to suppress the growth of true leaf primordia when seedlings on soil are exposed to drought (Lopez-Molina et al., 2001). However, it is unknown whether ABA would be required to suppress the growth of true leaf primordia in seedlings exposed to AIC stress. Therefore, we looked at *aba2-1* and *aba3-1*, mutants that synthesize less ABA due to different mutations in the ABA biosynthesis pathway (González-Guzmán et al.,

2002; Melotto et al., 2006). When grown under AIC stress, we found that both of those lines had a lower percentage of seedlings with primordia than WT (Figure 10). Alternatively, treatment of WT seedlings with ABA caused an increase in the percentage of seedlings with primordia, bringing it up to *mate45-1* levels (Figure 11). The WT phenotype was not significantly changed by other hormones that affect organogenesis (BAP and IBA) or auxin transport inhibitor (NPA). This suggests that the failure to arrest growth phenotype is dependent on the presence of ABA, and that the lack of ABA leads to more arrested growth.

After finding that the percentage of seedlings with primordia in WT was affected by ABA, we wanted to see if the *mate45-1* phenotype would also be affected by the amount of ABA in the seedling. To test this, we added fluridone, a chemical that blocks ABA synthesis, to seedlings growing under AIC stress. Figure 11 shows that the fluridone caused a reduction in the percentage of seedlings with primordia in *mate45-1* and *abcg25-5*, bringing them down to WT levels. It also caused a reduction in the percentage of seedlings with primordia in WT. Another way we tested this was by crossing *mate45-1* with *aba2-1*. We found that the percentage of *mate45-1 aba2-1* seedlings with primordia was similar to WT levels (Figure 10). This shows that the phenotype of *mate45-1* is indeed dependent on the amount of ABA and suggests that *MATE45* is somehow involved in the ABA pathway.

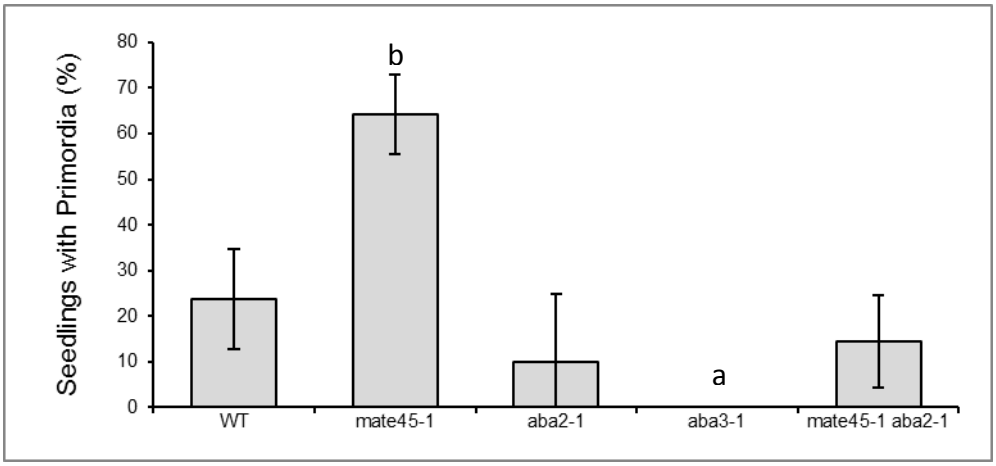

Figure 10. The percentage of seedlings with primordia when grown under AIC stress. The *aba* lines have reduced accumulation of ABA (Barrero et al., 2005). *mate45-1 aba2-1* is a cross that is homozygous for both the *mate45-1* and *aba2-1* mutations. The bars are denoted by "a" if significantly lower than WT and "b" if significantly higher than WT, based on a Student's t-test ($p < 0.05$).

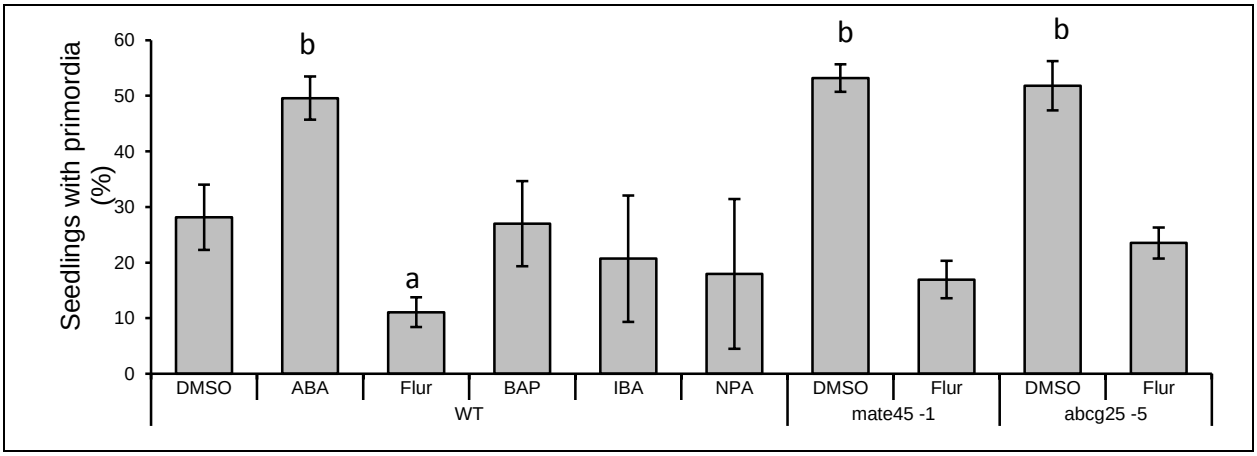

Figure 11. The percentage of seedlings with primordia when grown under AIC stress. DMSO was the solvent control. Fluridone is an ABA synthesis inhibitor, BAP is a synthetic cytokinin, IBA is an auxin, and NPA is an auxin transport inhibitor. After the seedlings had germinated, either 3µM of DMSO, BAP, IBA, NPA, or 10 µM of fluridone was added to the petri dish, then the seedlings were allowed to continue growing. The bars are denoted by "a" if significantly lower than the WT and "b" if significantly higher than the WT, based on a Student's t-test ($p < 0.05$).

<u>Dehydration analysis</u>

ABA has a large role in regulating the genes that respond to dehydration stress. ABA leads to stomata closure during drought conditions. Based on the observation that *mate45-1* and ABA affect primordia growth in AIC, we wanted to see if *mate45-1* also affected the

dehydration response. We indirectly measured the response to dehydration by measuring the amount of mass that the plants lost compared to the starting mass of the plant. This mass corresponded to the amount of water that was lost after five hours in dehydration conditions. As shown in Figure 12, *mate45-1*, *aba2-1*, and *abcg25-5* mutants lost significantly more water than WT. On the other hand, the *MATE45 mate45-1* line lost significantly less water than WT. The enhanced performance of *MATE45 mate45-1* could be due to the fact that its relative gene expression is slightly more than WT (Figure 8).

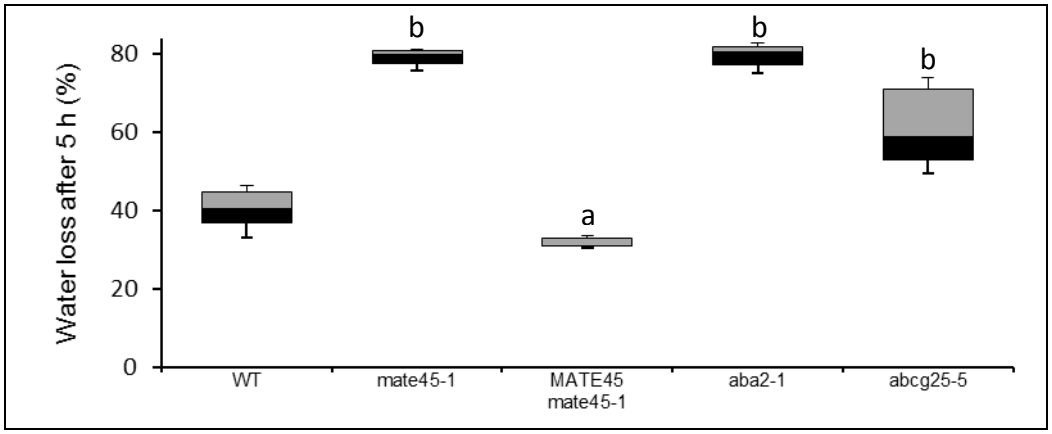

Figure 12. Plants that were grown on soil were excised at the rosette base, and were weighed every hour for 5 hours. The boxplot shows the percentage of water lost after 5h. The calculation for the percentage of water lost is in the materials and methods. The gray portion of each box is the third quartile and the black portion of each box is the second quartile. The bars are denoted by "a" if significantly lower than the WT control and "b" if significantly higher than the WT control, based on a Student's t-test ($p < 0.05$).

DISCUSSION

The *mate45-1* mutation led to an overexpression of transcripts of the *MATE45* gene, but created a truncated gene product. This mutation caused a failure to arrest true leaf primordia growth in seedlings growing in AIC beyond 10 days. The WT phenotype was rescued in *mate45-1* when a functional copy of *MATE45* was inserted (*MATE45 mate45-1*). This allowed us to confirm that the *mate45-1* phenotype we observed was indeed due to a mutation in the *MATE45* gene. When the *MATE45* gene was silenced in WT (*siMATE45-4*, *siMATE45-30*, and *siMATE45-31*), the percentage of seedlings with primordia was similar to *mate45-1*. This shows that the truncation of the mutant gene made a gene product that had reduced function, as if the transporter was being silenced. This is could be due to how the protein topology was altered after the 61 amino acid deletion, or that the deleted region was needed for the function of the protein. To determine whether the resulting phenotype was dominant or recessive, we looked at a line that was heterozygous for the *mate45-1* mutation (*mate45-1* (+/-)). The heterozygous line had a similar percentage of seedlings with primordia as the WT. This demonstrated that if there was some functional *MATE45*, it is enough to have the WT phenotype, as one copy is sufficient to suppress primordia growth. Therefore, the *mate45-1* mutation is recessive. This further supported that the failure to arrest growth phenotype of *mate45-1* was due to the truncation of the gene, rather than the overexpression, which would have been dominant.

It is thought that anthocyanins have a role in the plant's defense against abiotic stresses, and we hypothesized that *mate45-1* also had a role, and so we wanted to know whether the phenotype of *mate45-1* was caused by altered anthocyanin composition. We looked at the

percentage of seedlings with primordia in *tt4-11*, a mutant that does not synthesize anthocyanins, and found that they were similar to WT levels. And when we crossed *mate45-1* to *tt4-11*, we found that the percentage of seedlings with primordia were similar to *mate45-1* levels. The percentage of seedlings with primordia was not affected in the presence or absence of anthocyanins. This shows that the failure to arrest primordia growth of *mate45-1* is not dependent on altered anthocyanin profiles.

The failure to arrest true leaf growth in AIC was observed in both *abcg25-5*, an ABA transporter mutant, and *mate45-1*. As a result, we wanted to investigate whether there existed a relationship between the *mate45-1* mutation and ABA. Crossing *mate45-1* to the ABA synthesis mutant, *aba2-1*, reduced the percentage of seedlings with primordia to be the same as the WT. This demonstrated that the primordia growth of mate45-1 in AIC was ABA-dependent. This led us to believe that *MATE45* transports ABA, or is somehow involved in the regulation of the ABA pathway. We looked at the ABA synthesis mutants, *aba2-1* and *aba3-1*, and found that they had a lower percentage of seedlings with primordia than WT. This showed that the presence of ABA was responsible for the phenotype that we were seeing, and thus we would get the opposite phenotype in seedlings that had very low amounts of ABA. When we treated *mate45-1* and *abcg25-5* with fluridone, a chemical that blocks ABA synthesis, there was indeed a decrease in the percentage of seedlings with primordia, down to WT levels. On the other hand, when we treated WT with ABA, the percentage of seedlings with primordia increased to *mate45-1* levels. Overall, this suggested that the failure to arrest growth phenotype was dependent on the accumulation of ABA. Another possibility that explains this

phenotype is that the mutants had the same amount of ABA, but had different responses, perhaps because the ABA was not in the correct location.

In addition to the failure to arrest growth phenotype, we observed that *mate45-1* mutants lost more water than WT plants when subjected to dehydration conditions. The ABA synthesis and transporter mutants, *aba2-1* and *abcg25-5*, also lost more water than WT plants. In *aba2-1*, this phenotype can be explained by reduced levels of ABA in the plant, and in *abcg25-5*, by a problem with its transport to the guard cells. In *mate45-1*, we know that ABA is present in the plant, but there is a failure of ABA to activate stomata closure, perhaps because the ABA is not getting to the guard cells. When a functional *MATE45* gene is transformed into *mate45-1*, with slightly higher expression levels, there is less water loss, and the plant performs better than WT.

Overall, we found that *MATE45* was indeed involved in the plant's response to the abiotic stresses of AIC and dehydration. The *mate45-1* mutation caused a failure to arrest growth under AIC stress and increased water loss under dehydration stress. The ability to arrest growth in AIC was not affected by the anthocyanin profile of the plant, but was affected by the presence or the absence of ABA. Therefore, we believe that *MATE45* is involved in an ABA-dependent abiotic stress response pathway. In the future, we want to narrow down the role of *MATE45* in *Arabidopsis*, by determining its specific substrate and finding exactly where it fits into the ABA pathway. We could achieve this by performing transport assays in which we transform *MATE45* into an *Escherichia coli* background and measure its ability to transport ABA, precursors in the ABA pathway, and antagonists of ABA as well.

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Appendix A- Raw data for primordia experiments

Line	Number of seedlings with primordia	Number of total seedlings	Percentage of seedlings with promordia
WT	9	108	8.3
WT	29	82	35.4
WT	25	85	29.4
WT	20	146	13.7
WT	24	135	17.8
WT	25	95	26.3
WT	43	127	33.9
WT	41	106	38.7
mate45-1	43	78	55.1
mate45-1	48	98	49.0
mate45-1	41	84	48.8
mate45-1	66	113	58.4
mate45-1	79	114	69.3
mate45-1	68	96	70.8
mate45-1	65	115	56.5
abcg25-5	47	107	43.9
abcg25-5	57	105	54.3
abcg25-5	41	89	46.1
mate45 (+/-)	29	124	23.4
mate45 (+/-)	18	90	20.0
mate45 (+/-)	42	105	15.0
siMATE45-30	82	102	80.4
siMATE45-30	79	98	80.6
siMATE45-30	72	87	82.8
siMATE45-31	64	103	62.1
MATE45 mate45-1	23	91	25.3
MATE45 mate45-1	28	109	25.7
MATE45 mate45-1	18	65	27.7
MATE45 mate45-1	13	93	14.0
tt4-11	29	88	33.0
tt4-11	19	92	20.7
tt4-11	9	145	6.2
tt4-11	12	143	8.4
mate45-1 tt4-11	72	101	71.3
mate45-1 tt4-11	58	88	65.9
mate45-1 tt4-11	102	167	61.1
mate45-1 tt4-11	56	124	45.2
aba2-1	0	N/A	0.0

aba2-1	0	N/A	0.0
aba2-1	0	N/A	0.0
aba2-1	0	N/A	0.0
aba2-1	19	93	20.4
aba2-1	27	95	28.4
aba2-1	37	107	34.5
aba2-1	40	117	34.1
aba3-1	0	N/A	0.0
aba3-1	0	N/A	0.0
aba3-1	0	N/A	0.0
aba3-1	0	N/A	0.0
mate45-1 aba2-1	4	117	3.4
mate45-1 aba2-1	10	106	9.4
mate45-1 aba2-1	31	118	26.3
mate45-1 aba2-1	23	123	18.7
WT with DMSO	30	92	32.6
WT with DMSO	28	123	22.8
WT with DMSO	38	145	26.2
WT with DMSO	44	126	34.9
WT with DMSO	30	92	32.6
WT with DMSO	28	123	22.8
WT with DMSO	38	145	26.2
WT with DMSO	44	126	34.9
WT with ABA	58	114	50.9
WT with ABA	70	133	52.6
WT with ABA	66	146	45.2
WT with Fluridone	38	84	45.2
WT with Fluridone	40	132	30.3
WT with Fluridone	23	113	20.4
WT with IBA	7	127	5.5
WT with IBA	16	135	11.9
WT with IBA	36	143	25.6
WT with IBA	32	107	29.8
WT with IBA	26	84	30.7
WT with BAP	36	95	37.9
WT with BAP	34	127	26.8
WT with BAP	30	137	21.9
WT with BAP	24	112	21.4
WT with NPA	10	162	5.9
WT with NPA	27	174	15.6
WT with NPA	38	117	32.5

APPENDIX B- Raw data for dehydration experiment

WT

	Mass of plant						
Time (h)	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
0	0.7304	0.6906	0.7369	0.7423	0.8306	0.4335	0.4877
0.5	0.6195	0.5768	0.6437	0.6599	0.7093	0.3656	0.4178
1	0.59	0.5506	0.6139	0.6248	0.6775	0.3429	0.3914
1.5	0.5674	0.5304	0.5906	0.5983	0.6538	0.326	0.3699
2	0.5473	0.5121	0.5119	0.5736	0.6332	0.3089	0.3508
2.5	0.5282	0.5483	0.4939		0.613	0.2931	0.3323
3	0.5107	0.5293	0.4776		0.5939	0.2792	0.3163
5	0.4521	0.4613	0.423		0.5276	0.2325	0.2655
8	0.3802	0.377	0.356		0.4439	0.1785	0.2099

mate45-1

	Mass of plant						
Time (h)	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
0	0.6662	0.4194	0.4112	0.1399	0.6262	0.7016	0.6646
0.5	0.4914	0.299	0.2863	0.0926	0.453	0.5091	0.468
1	0.408	0.2456	0.2269	0.0723	0.3778	0.4295	0.3852
1.5	0.3493	0.2061	0.1861	0.059	0.3255	0.3752	0.3273
2	0.3016	0.1754	0.1533	0.0493	0.2836	0.3313	0.2773
2.5		0.1492	0.1274	0.0422	0.246	0.2923	0.2386
3		0.1278	0.1089	0.0369	0.2149	0.2589	0.2081
5		0.0838	0.0778	0.0266	0.1466	0.1713	0.1348

MATE45 mate45-1

	Mass of Plant							
Time(h)	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8
0	1.1716	1.2158	1.2293	1.0978	1.3382	1.0938	0.986	0.9758
0.5	1.0983	1.1394	1.1526	1.0157	1.2566	1.0224	0.9218	0.914
1	1.0464	1.0862	1.0989	0.965	1.1989	0.9746	0.8767	0.8718
1.5	1.0103	1.0512	1.0645	0.9313	1.1614	0.9432	0.846	0.8437
2	0.976	1.0177	1.0312	0.8992	1.1258	0.914	0.8162	0.8171
2.5	0.9419	0.9845	0.9981	0.868	1.0907	0.885	0.7877	0.7912
3	0.9081	0.9529	0.966	0.8386	1.0562	0.8574	0.7602	0.766
5	0.7904	0.8387	0.8478	0.7295	0.93	0.756	0.6594	0.6781

aba2-1

	Mass of plant						
Time (h)	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
0	0.2388	0.1636	0.148	0.2039	0.2315	0.1062	0.2176
0.5	0.1405	0.1069	0.1025	0.1157	0.1366	0.0657	0.1252
1	0.0993	0.0848	0.0851	0.0817	0.0963	0.0536	0.083
1.5	0.0769	0.0698	0.0732	0.0647	0.0759	0.0461	0.0633
2	0.0648	0.0592	0.0646	0.0555	0.0658	0.0408	0.0543
2.5	0.0569		0.0575	0.0509	0.0593	0.036	0.0496
3	0.0509		0.0516	0.0475	0.0546	0.0329	0.0465
5	0.0408		0.0368	0.0404	0.044	0.0251	0.0394

abcg25-5

	Mass of plant						
Time (h)	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
0	0.1718	0.1434	0.3954	0.0665	0.3904	0.0824	0.3813
0.5	0.136	0.1141	0.3344	0.0484	0.3218	0.0597	0.3167
1	0.1276	0.1049	0.3162	0.044	0.3025	0.053	0.2954
1.5	0.1216	0.0983	0.3032	0.041	0.2887	0.0489	0.2806
2		0.0923	0.2911	0.0381	0.2761	0.0455	0.2676
2.5		0.0873	0.2797	0.0355	0.2651	0.0429	0.2564
3		0.0822	0.2687	0.0329	0.2544	0.0402	0.2451
5		0.0666	0.2321	0.0255	0.2194	0.0321	0.211